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Original research

Pulsed electromagnetic fields accelerate functional recovery of transected sciatic nerve bridged by chitosan conduit: An animal model study

Rahim Mohammadi [a, *], Darab Faraji [b], Hanieh Alemi [b], Aram Mokarizadeh [c]

[a] Department of Clinical Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[b] Department of Surgery, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
[c] Department of Immunology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran

HIGHLIGHTS

- Effect of body exposure to pulsed electromagnetic fields on transected nerve regeneration was assessed.
- Body exposure to PEMF improved functional recovery and morphometric indices of sciatic nerve.
- PEMF combined with chitosan grafting could be an effective, safe and tolerable treatment in clinical practice.

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ABSTRACT

Introduction: Effect of whole body exposure to pulsed electromagnetic fields (PEMF) on nerve regeneration in a rat sciatic nerve transection model was assessed. **Methods**: Sixty male white Wistar rats were divided into four experimental groups ($n = 15$), randomly: In transected group (TC) left sciatic nerve was transected and stumps were fixed in adjacent muscle. In chitosan group (CHIT) the defect was bridged using a chitosan conduit filled with phosphate-buffered saline. In treatment group (CHIT/PEMF) the whole body was exposed to PEMF (0.3 mT, 2 Hz) for 4 h/day within 1–5 days. In normal control group (NC) sciatic nerve was only dissected and manipulated. Each group was subdivided into three subgroups of five animals each and nerve fibers were studied 4, 8 and 12 weeks after surgery. **Results**: Behavioral, functional, electrophysiological, biomechanical, gastrocnemius muscle mass findings and morphometric indices confirmed faster recovery of regenerated axons in CHIT/PEMF than in CHIT group ($p < 0.05$). Immunohistochemical reactions to S-100 in CHIT/PEMF were more positive than that in CHIT group. **Discussion**: Whole body exposure to PEMF improved functional recovery and morphometric indices of sciatic nerve. Detailed mechanism of neuroprotective action remains to be investigated. **Conclusion**: PEMF combine with chitosan grafting could be considered as an effective, safe and tolerable treatment for peripheral nerve repair in clinical practice.

1. Introduction

Pronounced improvements in the diagnosis and repair of transected peripheral nerves are results of technological advances in diagnostic imaging, neurosurgical instrumentation and the use of a surgical microscope [1]. The ideal surgical repair technique should accomplish good wound healing with minimal scar formation and direct the nerve sprouts into their correct targets [2]. The conduits act to guide axons sprouting from the regenerating nerve end, provide a microenvironment for diffusion of neurotrophic and neurotropic factors secreted by the injured nerve stump, as well as help protect from infiltration of fibrous tissue [3].

Different graft equivalents have also been applied to bridge the nerve stump and regulated through the interaction of a variety of protein and cell signals [4]. Biodegradable nerve guides as a temporary scaffold are better than non-degradable biomaterials because the latter remain in situ as a foreign body and ultimately result in limiting recovery of nerve function [5]. Nevertheless, the

* Corresponding author. Department of Clinical Sciences, Faculty of Veterinary Medicine, Urmia University, Nazloo Road, Urmia, 57153 1177, Iran.
E-mail address: r.mohammadi@urmia.ac.ir (R. Mohammadi).

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resistance to biodegradation can be a cause of chronic nerve compression in the long run and a second surgery may therefore be required for its removal. Beneficial effects of chitosan as a conduit in promoting nerve regeneration have already been documented and it seems chitosan as a natural polymer has excellent properties including biocompatibility, biodegradability, non-toxicity and adsorption properties, and might be a suitable functional material for peripheral nerve regeneration [6–8].

Pulsed electromagnetic fields (PEMF) are reported to promote peripheral nerve regeneration to an extent similar to that observed with conditioning lesions, growth factors, and hormones [9]. Exposure to PEMF as a pretreatment prior to crush injury has resulted in acceleration of axonal regrowth, and consistent with the stimulation of regenerative neurite outgrowth increased functional outcomes such as walking behavior [10–13]. PEMF has also been shown to promote neurite outgrowth *in vitro* [14].

Others have demonstrated that prolonged PEMF regimen had led to delayed histological peripheral nerve regeneration and increased oxidative stress but no loss of function recovery [15].

These contradictory results were probably due to technical differences, specifically to different protocols for PEMF exposure. Therefore, the present investigators concluded that the issue was not clear and that more experiments were needed to assess the possible benefits of PEMF exposure on peripheral nerve regeneration.

Furthermore, Promising results regarding the beneficial effect of PEMF on transected peripheral nerve regeneration are poor and not supported by functional tests, to the best of knowledge of the authors, which play a crucial role in the assessment of functional nerve recovery.

The present study was conducted to study functional effects of PEMF on peripheral nerve regeneration. Assessment of the nerve regeneration was based on behavioral, functional, electrophysiological, biomechanical, histomorphometric and immunohistochemical (Schwann cell detection by S-100 expression) criteria 4, 8 and 12 weeks after surgery.

2. Materials and methods

2.1. Study design and animals

Sixty male white Wistar rats weighing approximately 290 g were divided into four experimental groups ($n = 15$), randomly: In transected group (TC) left sciatic nerve was transected and stumps were fixed in adjacent muscle. In chitosan group (CHIT) the defect was bridged using a chitosan conduit filled with phosphate-buffered saline. In treatment group (CHIT/PEMF) the whole body was exposed to PEMF (0.3 mT, 2 Hz) for 4 h/day within 1–5 days. In normal control group (NC) sciatic nerve was only exposed and manipulated. Each group was further subdivided into three subgroups of five animals each and surveyed 4, 8 and 12 weeks after surgery. Two weeks before and during the experiments, the animals were housed in individual plastic cages with an ambient temperature of (23 ± 3) °C, stable air humidity and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. All measurements were made by two blinded observers unaware of the analyzed groups.

2.2. Preparation of chitosan conduit

Chitosan solution was prepared dissolving medium molecular weight, crab shell chitosan (~400 kDa, 85% deacetylated) (Fluka, Sigma–Aldrich St. Louis, MO, USA) in an aqueous solution (1% v/v) of glacial acetic acid (Merck, Darmstadt, Germany) to a concentration of 2% (w/v) while stirring on a magnetic stirrer-hot plate. The solution was stirred with low heat (at 50 °C) for 3 h. The resultant chitosan solution was filtered through a Whatman No. 3 filter paper then vacuum filtration to remove any un-dissolved particles. To overcome the fragility of chitosan, glycerol (Sigma Chemical Co., St. Louis, MO, USA) was added as 30% (w/w) of the total solid weight in solution [16]. Chitosan conduit was made according to the method described by others24 by gentle injection of the prepared solution into a home-made mold. The prepared conduit was 2 mm in external diameter, 1.8 mm in internal diameter and 10 mm in length. This internal diameter complies with optimal function in rat models [17].

2.3. Surgical procedure

Animals were anesthetized by intraperitoneal administration of ketamine–xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg). The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain [18]. The University Research Council approved all experiments.

Following surgical preparation in the normal control group, the left sciatic nerve was exposed through a gluteal muscle incision and after careful homeostasis the muscle was sutured with resorbable 4/0 sutures, and the skin with 3/0 nylon. In TC group, the left sciatic nerve was transected proximal to the tibio-peroneal bifurcation where a 7 mm segment was excised, leaving a 10 mm gap due to retraction of nerve ends. Proximal and distal stumps were fixed in the adjacent muscle with 10/0 nylon epineurial suture. No graft was interposed between the stumps. In the CHIT group, a 7-mm nerve segment was resected to produce a 10 mm nerve gap after retraction of the nerve transected ends. The gap was bridged using a chitosan conduit. Two 10/0 nylon sutures were used to anchor the graft to the epineurium at each end. The animals were anesthetized and euthanized with transcardiac perfusion of a fixative containing 2% paraformaldehyde and 1%glutaraldehyde buffer (pH 7.4) 4, 8 and 12 weeks after surgery.

2.4. Pulsed electromagnetic fields treatment

Following recovery from anesthesia, rats were randomly assigned to control or experimental groups. Pulsed electromagnetic fields treatment was performed based on a method described by others [10,11]. In brief, on days 1–5, each animal was placed in an all-plastic restrainer located between Helmholtz coils and treated for 4 h each day with the PEMF signal generator either activated (CHIT/PEMF group) or not activated (CHIT). PEMF was applied using paired Helmholtz coils (PHYWE, 06514, Germany) 30 cm in diameter, placed 15 cm apart. When connected to a signal generator (Funktiongenerator, PHYWE, Göttingen, Germany), these coils produced a magnetic field amplitude of 0.3 mT with a pulse duration of 20 msec, repeated at a pulse repetition rate of 2 Hz. The rise time was 0.85 msec, the fall time 0.68 msec.

2.5. Behavioral testing

Functional recovery of the nerve was assessed using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale for rat hind limb motor function [19]. Although BBB is widely used to assess functional recovery in spinal cord injured animals, however, it has been demonstrated that it could be most useful in assessment of never repair processes in peripheral nerve injuries [20]. Scores of 0 and 21 were given when there were no spontaneous movement and normal movement, respectively. A score of 14 shows full weight support and complete limbs coordination. BBB recordings were performed by a trained observer who was blinded to the

experimental design. The testing was performed in a serene environment. The animals were observed and assessed within a course of a 4-min exposure to an open area of a mental circular enclosure. BBB scores were recorded once before surgery in order to establish a baseline control and again weekly thereafter to assess functional recovery during 12 weeks.

3. Functional assessment of reinnervation

3.1. Sciatic functional index (SFI)

Walking track analysis was performed 4, 8 and 12 weeks after surgery based on the method of others [21]. The lengths of the third toe to its heel (PL), the first to the fifth toe (TS), and the second toe to the fourth toe (IT) were measured on the experimental side (E) and the contralateral normal side (N) in each rat. The sciatic function index (SFI) of each animal was calculated by the following formula:

$$\mathrm{SFI} = -38.3 \times (\mathrm{EPL} - \mathrm{NPL})/\mathrm{NPL} + 109.5 \times (\mathrm{ETS} - \mathrm{NTS})/\mathrm{NTS} + 13.3 \times (\mathrm{EIT} - \mathrm{NIT})/\mathrm{NIT} - 8.8$$

In general, SFI oscillates around 0 for normal nerve function, whereas around −100 SFI represents total dysfunction. SFI was assessed in the NC group and the normal level was considered as 0. SFI was a negative value and a higher SFI meant the better function of the sciatic nerve.

3.2. Static sciatic index (SSI)

SSI is a time-saving digitized static footprint analysis described by others [22]. A good correlation between the traditional SFI and the newly developed static sciatic index (SSI) and static toe spread factor (TSF), respectively, has been reported by others [22]. The SSI is a time-saving and easy technique for accurate functional assessment of peripheral nerve regeneration in rats and is calculated using the static factors, not considering the print length factor (PL), according to the equation:

$$\mathrm{SSI} = [(108.44 \times \mathrm{TSF}) + (31.85 \times \mathrm{ITSF})] - 5.49$$

Where:

$$\mathrm{TSF} = (\mathrm{ETS} - \mathrm{NTS})/\mathrm{NTS}$$

$$\mathrm{ITSF} = (\mathrm{EIT} - \mathrm{NIT})/\mathrm{NIT}$$

Like SFI, an index score of 0 was considered normal and an index of −100 indicated total impairment. When no footprints were measurable, the index score of −100 was given.

3.3. Electrophysiological assessment

At the end of the study period, following walking track, all animals were subjected to electrophysiological studies using Nacro bio system 320-3760 A trace 80 (USA). Under general anesthesia, the left sciatic nerve was re-exposed by incision of the skin at the previous surgical site. Single electrical pulses at supramaximal intensity were delivered via bipolar electrodes placed in turn at the proximal and distal trunk of the grafted nerve and EMG was recorded by inserting an electrode into the belly of gastrocnemius muscle.

The difference in latency of EMG was measured, and the distance between the proximal and distal sites of stimulation was measured to calculate the conduction velocity across the regenerated tissue cable. On the contralateral side of each animal similar measurement was made for determination of conduction velocity. The conduction velocity of the bridged nerve was expressed as a percentage of that on the intact side of each animal to cancel off variations between animals (% CVR) [23].

3.4. Biomechanical testing

Following electrophysiological assessments the regenerated nerves were harvested and placed in a normal saline bath at room temperature. The samples were then fixed between frozen fixtures in a mechanical apparatus. The TA.XTPlus Texture Analyzer mechanical test device was used for the assessment (Stable Micro Systems, Surrey GU7 1YL, UK). After 5 min, the frozen fixtures were tightened to ensure that no slippage occurred during testing. The initial length was set to 10 mm. Each sample was stretched at a constant rate of 1 mm/min. The load and displacement were sampled 5 times per second. Each sample was stretched to complete tensile failure. Samples were kept wet moist during testing using a drop of normal saline solution to the nerve segments.

3.5. Muscle mass

Recovery assessment was also indexed using the weight ratio of the gastrocnemius muscles 12 weeks after surgery. Immediately after sacrificing of animals, gastrocnemius muscles were dissected and harvested carefully from intact and injured sides and weighed while still wet, using an electronic balance.

3.6. Histological preparation and morphometric studies

Proximal nerve segments were detached from mechanical device and fixed with glutaraldehyde 2.5%. They were post fixed in OsO4 (2%, 2 h), dehydrated through an ethanol series and embedded in paraffin. The nerves were cut in 5 μm in the middle, stained with toluidine blue and examined under light microscopy. Morphometric analysis was carried out using an image analyzing software (Image-Pro Express, version 6.0.0.319, Media Cybernetics, Silver Springs, MD, USA). Equal opportunity, systematic random sampling and two-dimensional dissector rules were followed in order to cope with sampling-related, fiber-location-related and fiber-size related biases [24].

3.7. Immunohistochemical analysis

In this study, anti-S-100 (1:200, DAKO, USA) was used as marker for myelin sheath. Specimens were post fixed with 4% paraformaldehyde for 2 h and embedded in paraffin. Prior to immunohistochemistry nerve sections were dewaxed and rehydrated in PBS (pH 7.4). Then the nerve sections were incubated with 0.6% hydrogen peroxide for 30 min. To block non-specific immunoreactions the sections were incubated with normal swine serum (1:50, DAKO, USA). Sections were then incubated in S-100 protein antibody solution for 1 h at room temperature. They were washed three times with PBS and incubated in biotynilated anti-mouse rabbit IgG solution for 1 h. Horseradish peroxidase-labeled secondary antibody was applied for 1 h. After that all sections were incubated with 3,3′- diaminobenzidine tetrahydrochloride chromogene substrate solution (DAB, DAKO, USA) for 10 min. The results of immunohistochemistry were examined under a light microscope.

3.8. Statistical analysis

The results were expressed as means ± SD. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Model assumptions were evaluated by examining the residual plot. Results were analyzed using a factorial ANOVA with two between-subjects factors. Bonferroni test for pairwise comparisons was used to examine the effect of time and treatments. The differences were set at $P < 0.05$.

4. Results

4.1. Behavioral testing

4.1.1. BBB recovery

In order to assess hind limb recovery the open field locomotor was used. Fig. 1 shows BBB scores compared to the baseline. All experimental groups, except for NC, showed the greatest degree of functional deficit one week after surgery. The CHIT/PEMF group showed significant improvement in locomotion of the operated limb compared to the CHIT group during the study period ($P < 0.05$).

4.2. Recovery of sciatic nerve function

4.2.1. SFI outcome

Fig. 2 shows sciatic function index (SFI) values in all four experimental groups. Prior to surgery, SFI values in all groups were near zero. After the nerve transection, the mean SFI decreased to −100 due to the complete loss of sciatic nerve function in all animals. At the end of the study period, animals of CHIT/PEMF group achieved a mean value for SFI of −36.6 ± −3.19 whereas in group CHIT a mean value of −44.2 ± −4.11 was found. The statistical analyses revealed that the recovery of nerve function was significantly ($P < 0.05$) different between CHIT/PEMF and CHIT groups and exposure to the PEMF in significantly accelerated functional recovery in the course of time.

4.2.2. SSI outcome

Changes in SSI were similar to those observed in SFI, indicating significant deficit following the sciatic nerve transection (Fig. 3). Changes in SSI were significant at weeks 4, 8 and 12 of recovery ($P < 0.05$).

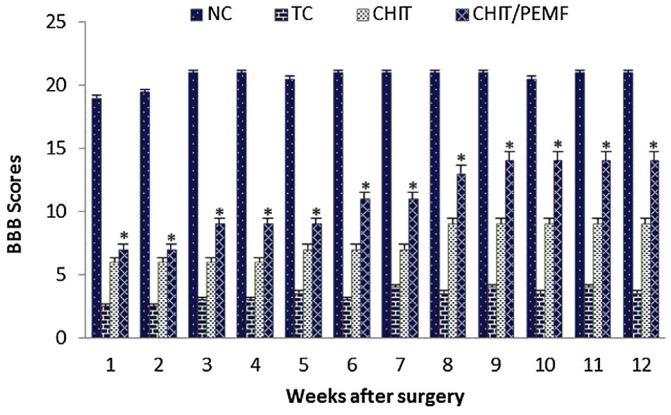

Fig. 1. BBB score for all experimental groups. PEMF with chitosan grafting gave better scores than in CHIT group. Standard error at each data point is shown with bars. *$P < 0.05$ vs CHIT group.

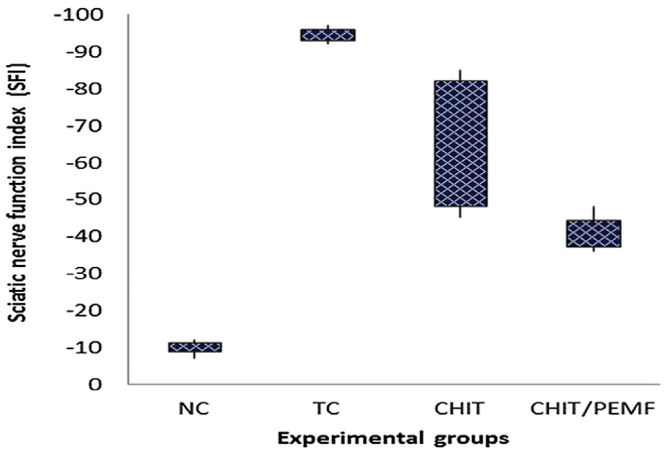

Fig. 2. Box-and-whisker plots of sciatic nerve function index values in each experimental group during the study period. PEMF with chitosan grafting gave better results in functional recovery of the sciatic nerve than in CHIT group.

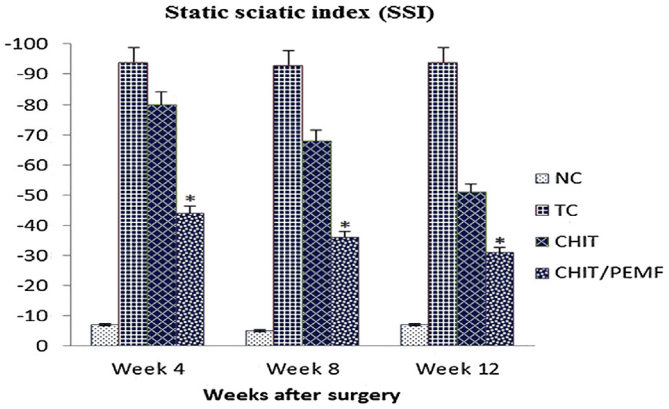

Fig. 3. Bar graph indicating static sciatic index (SSI) values in each experimental group during the study period. PEMF with chitosan grafting gave better results in functional recovery of the sciatic nerve than in CHIT group. Data are presented as mean ± SD. *$P < 0.05$ *vs* CHIT group.

4.3. Electrophysiological measurement

Figs. 4 and 5 show nerve conduction velocity (NCV) along regenerated sciatic nerves in experimental groups. NCV in CHIT/PEMF

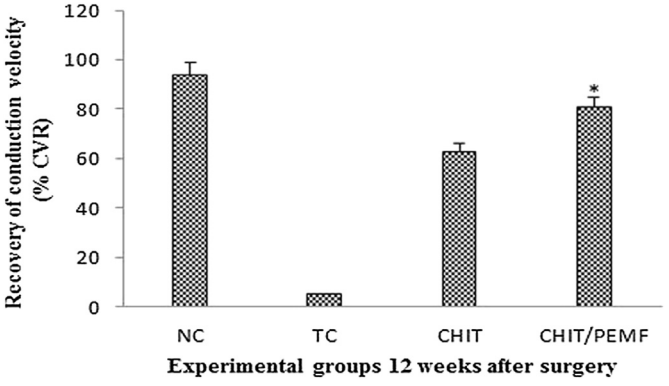

Fig. 4. Percentage recovery of conduction velocity in experimental groups. Nerve conduction velocity in PEMF treated animals was significantly higher than that in CHIT group. Data are presented as mean ± SD. *$P < 0.05$ vs CHIT group.

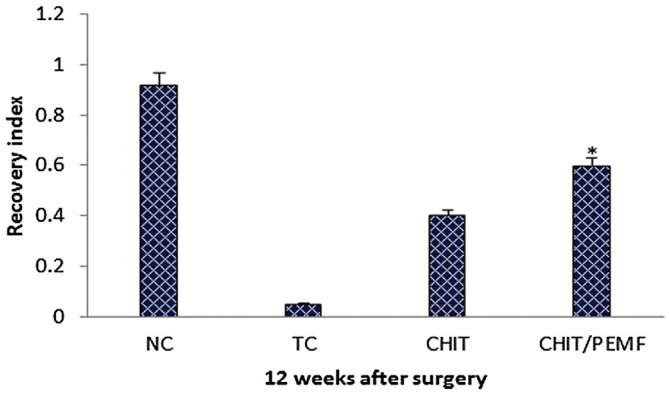

Fig. 5. Recovery index in experimental groups. Recovery index in PEMF treated animals was significantly better than that in CHIT group. Data are presented as mean ± SD. *$P < 0.05$ vs CHIT group.

group was significantly higher than that in the CHIT group ($P < 0.05$).

4.4. Biomechanical measurements

Maximum pull force (F_{max}) of normal sciatic nerve was found to be 5.56 ± 0.43. F_{max} of nerve samples in experimental groups are shown in Table 1. F_{max} in CHIT/PEMF group (3.94 ± 0.18) was significantly higher than that in the CHIT group (2.68 ± 0.21) ($P < 0.05$). Tensile strength, the amount of force per unit of initial cross-sectional area at tensile failure, was measured based on F_{max} and nerve cross sectional area. 12th week assessment revealed tensile strength of regenerated nerves exposed to PEMF (5.46 ± 0.19) was higher than those in the CHIT group (4.33 ± 0.22) ($P < 0.05$). Ultimate strain, the amount of elongation divided by the initial specimen length achieved at the point of tensile failure, in CHIT/PEMF group (0.39 ± 0.03)was significantly higher than that in the CHIT group (0.31 ± 0.03) ($P < 0.05$). Toughness, reflecting the properties of anti-deformation and anti-fracture of nerve, was determined by the nerve itself and could reflect "looseness" or "toughness" of nerve. Toughness in CHIT/PEMF group (0.81 ± 0.16) was significantly higher than that in the CHIT group (0.51 ± 0.14) ($P < 0.05$).

4.5. Muscle mass measurement

The mean ratios of gastrocnemius muscle weight were measured at the end of the study period. There was a statistically significant difference between the muscle weight ratios of the CHIT/PEMF and CHIT groups ($P < 0.05$). The results showed that in the CHIT/PEMF group, the muscle weight ratio was larger than in the CHIT group, and weight loss in the gastrocnemius muscle was ameliorated by exposure to PEMF (Fig. 6).

Table 1
Biomechanical analyses of regenerated nerves for each of the experimental groups: values are given as mean ± SD.

Groups	Maximum pull force (N)	Tensile strength (MPa)	Ultimate strain	Toughness (N/mm)
TC	1.41 ± 0.19	2.34 ± 0.33	0.12 ± 0.02	0.26 ± 0.08
NC	5.56 ± 0.43	7.37 ± 1.25	0.56 ± 0.04	1.27 ± 0.53
CHIT	2.68 ± 0.21	4.33 ± 0.22	0.31 ± 0.03	0.51 ± 0.14
CHIT/PEMF	3.94 ± 0.18[a]	5.46 ± 0.19[a]	0.39 ± 0.03[a]	0.81 ± 0.16[a]

[a] The mean difference is significant at the 0.05 level *vs*. SIL group.

4.6. Histological and morphometric findings

The CHIT/PEMF group presented significantly greater nerve fiber, axon diameter, and myelin sheath thickness during study period, compared to CHIT animals ($P < 0.05$). Normal control group presented significantly greater nerve fiber and axon diameter, and myelin sheath thickness compared to CHIT/PEMF and CHIT groups animals (Figs. 7–10). In case of myelin thickness there was no significant difference between CHIT/PEMF and CHIT groups, morphometrically ($P > 0.05$).

4.7. Immunohistochemistry

Immunoreactivity to S-100 protein was extensively observed in the cross sections of regenerated nerve segments. The expression of S-100 protein signal was located mainly in the myelin sheath. The axon also showed a weak expression indicating that Schwann cell-like phenotype existed around the myelinated axons (Fig. 11). In both CHIT/PEMF and CHIT groups, the expression of S-100 and the findings resembled those of the histological evaluations.

5. Discussion

A promising alternative to autologous nerve grafting could be entubulation neurorrhaphy [25]. In the present study we used chitosan as a nerve guide. Selection of an appropriate method to evaluate functional recovery of nerve regeneration is extremely influential. The results of the present study showed that PEMF exposure resulted in faster functional recovery of the sciatic nerve during the study period. Information taken from BBB scale may be invaluable in evaluation of peripheral nerve process. Results of the present study showed that the PEMF treated animals had been improved in locomotion of the operated limb compared to the CHIT group during the study period. Walking track analysis has frequently been used to reliably determine functional recovery following nerve repair in rat models [21,26]. Nerve conduction measurement is a direct evidence for the study of nerve transmission [27]. The conduction velocity depends on the diameter of axons and the thickness of myelin sheath [28]. The results of the present study showed significantly different conduction velocity between the PEMF treated animals and CHIT bridged regenerated sciatic nerves, therefore, the CHIT conduit in combination with

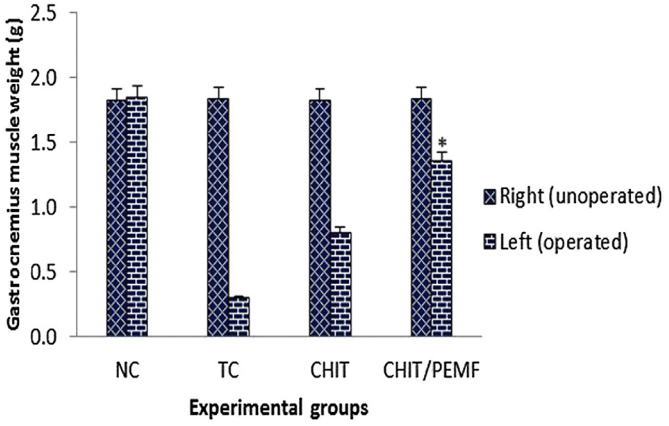

Fig. 6. Gastrocnemius muscle weight measurement. The gastrocnemius muscles of both sides (operated left and unoperated right) were excised and weighed in the experimental groups at 12 weeks after surgery. Data are presented as mean ± SD. *$P < 0.05$ *vs* CHIT group.

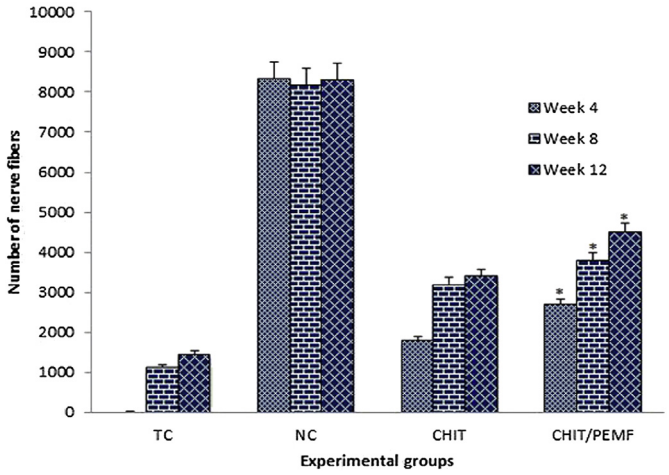

Fig. 7. The graph shows the quantitative results of fiber counting. The mean number of nerve fibers in NC group was nearly 8425 ± 241 (mean ± SD). Both groups of CHIT and CHIT/PEMF showed the lower number of fibers than the NC group even at the end of the study period.

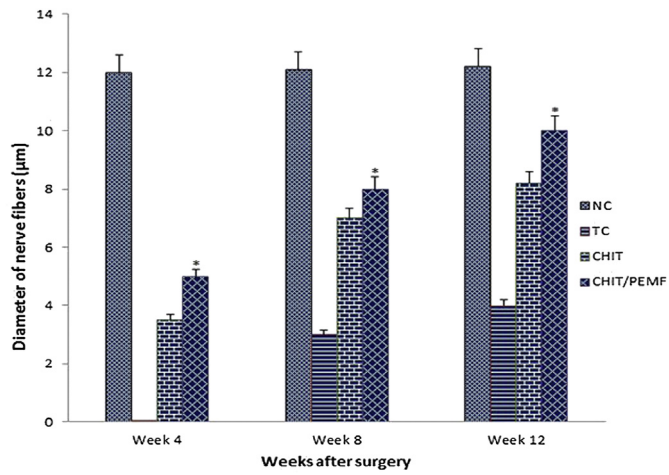

Fig. 8. The graph shows the quantitative results of mean diameter of nerves fibers. The mean diameter of nerve fibers in NC group was nearly 12.4 ± 0.14 (mean ± SD). Both groups of CHIT and CHIT/PEMF showed the lower mean diameter of nerve fibers than the NC group even at the end of the study.

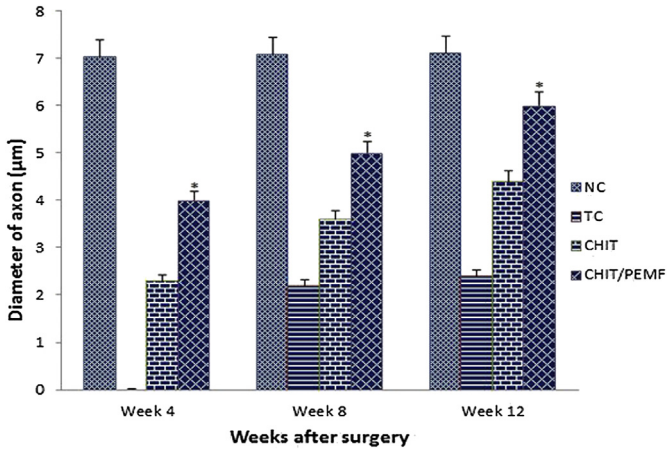

Fig. 9. The graph shows the quantitative results of mean diameter of axons. The mean diameter of axons in NC group was nearly 7.4 ± 0.12 (mean ± SD). Both groups of CHIT and CHIT/PEMF showed the lower mean diameter of axons than the NC group even at the end of the study period.

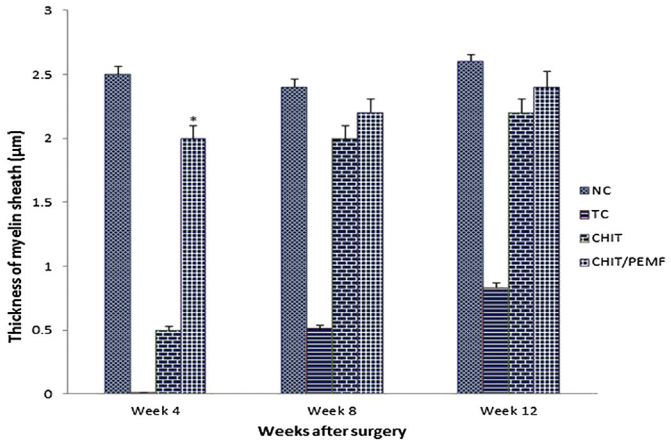

Fig. 10. The graph shows the quantitative results of mean thickness of myelin sheath. The mean thickness of myelin sheath in NC group was nearly 2.5 ± 0.06 (mean ± SD). Both groups of CHIT and CHIT/PEMF showed the lower mean diameter of axons than the NC group even at the end of the study period.

PEMF could be assumed as a safe technique with no nerve conduction interference.

PEMF exposure to regenerated nerve in the present study resulted in the enhanced biomechanical indices that were in agreement with morphometric findings. Left gastrocnemius muscle weight was significantly greater in the CHIT/PEMF group than in the CHIT group, indicating indirect evidence of successful end organ reinnervation in the PEMF treated animals.

It has been demonstrated that morphometric indices are measures of regenerated nerve maturity and quality of regeneration [29]. Larger diameters of axons and thicker myelination give rise to improved nerve function compared to smaller and thinner myelinated fibers [30]. At week 12 quantitative morphometrical indices of regenerated nerve fibers showed significant differences between the CHIT and CHIT/PEMF groups, indicating a beneficial effect of PEMF on the nerve regeneration.

In immunohistochemistry the expression of myelin sheath special proteins was evident in both groups which indicate the normal histological structure. The location of reactions to S-100 in the PEMF/CHIT group was clearly more marked than in the CHIT group implying that both regenerated axon and Schwann cell-like cells existed and were accompanied by the process of remyelination and the structural recovery of regenerated nerve fibers.

Although both morphological and functional data have been used to assess neural regeneration after induced crush injuries, the correlation between these two types of assessment is usually poor [31–33]. Classical and newly developed methods of assessing nerve recovery, including histomorphometry, retrograde transport of horseradish peroxidase and retrograde fluorescent labeling do not necessarily predict the reestablishment of motor and sensory functions [26,32,34–36]. Although such techniques are useful in studying the nerve regeneration process, they generally fail in assessing functional recovery [32]. Therefore, research on peripheral nerve injury needs to combine both functional and morphological assessment. It has been demonstrated that arrival of sprouts from the proximal stump at the distal nerve stump does not necessarily imply recovery of nerve function [36].

The effect of PEMF on cells and organisms after short-term exposure has been reported in many studies. There are many potential mechanisms by which PEMF might affect neurotrophic

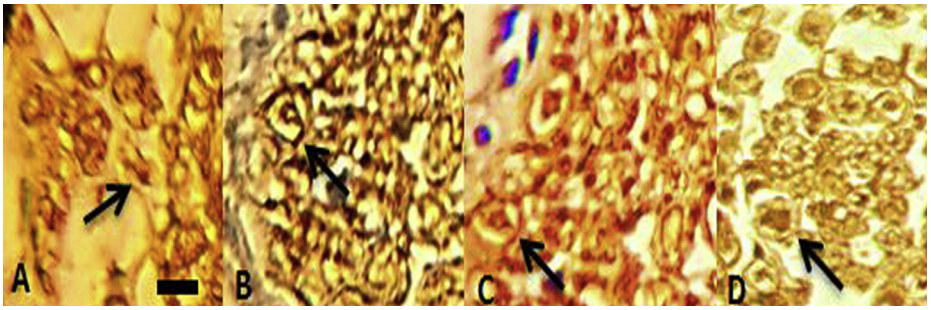

Fig. 11. Immunohistochemical analysis of the regenerated nerves 12 weeks after surgery from TC (A), CHIT (B), CHIT/PEMF(C) and NC (D) groups. There is clearly more positive staining of the myelin sheath-associated protein S-100 (arrows) within the periphery of nerve, indicating well organized structural nerve reconstruction in PEMF treated nerve compared to that of the CHIT. Scale bar: 10 μm.

factor levels in nerve tissue. The absence of PEMF effects in nerve segments isolated from non-transected rats raises the possibility that these mechanisms occur primarily in injured rather than in normal animals. Previous studies have demonstrated that at 6 h post-transection, increased levels of NGF in distal segments resulted from blocked retrograde transport rather than local synthesis [37]. Thus, the significant effect of PEMF on reducing nerve growth factor-like activity and levels as early as 6 h post-transection suggested that PEMF acts via mechanisms distinct from synthesis of nerve growth factor or nerve growth factor-like factors. Other growth factors potentially influence nerve regeneration and through which PEMF might act include brain derived neurotrophic factor, ciliary neurotrophic factor insulin-like growth, fibroblast growth factor, and glia-derived neurotrophic factor [38–41]. Cellular mechanisms underlying all these magnetic stimulations remain unclear. Although the positive influence of the fields is more and more recognized and used in therapeutic applications, the general effectiveness is still controversial. There are obvious knowledge gaps that make a conclusion of the risk for neurodegenerative diseases due to magnetic fields exposure very difficult [42].

Experimental research efforts should include a proper long-term perspective, possibly as life-long animal studies. Comprehensive and systematic studies regarding threshold identification as well as studies with non-activated and pre-activated cells could give more insight into the mode of action of field exposure and cells [42].

Even though our preliminary study shows the neuroprotective action of local PEMF in peripheral nerve injuries, determining the molecular mechanisms leading to the neuroprotective action remains needs to be investigated. We have not given the histological and molecular evidence for neuroprotective action of PEMF. This may be considered as a limitation to our study.

Therefore, the authors stress that the aim of the current investigation was to evaluate whole body exposure and clinical treatment potential of PEMF on transected nerve regeneration including functional assessments of the nerve repair, a case not considered in previous studies. The results of the present study indicated that whole body exposure to PEMF could be of benefit after chitosan conduit tubulization. Detailed mechanism of neuroprotective action remains to be investigated.

6. Conclusion

In conclusion results of the present study demonstrated that whole body exposure to PEMF improved functional recovery and morphometric indices of transected sciatic nerve. PEMF could be considered as an effective, safe and tolerable treatment for peripheral nerve repair in clinical practice.

Ethical approval

The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain* and the University Research Council approved all experiments.

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Author contribution

Rahim Mohammadi: Study design and writing.

Darab Faraji and Hanieh Alemi: Grafting procedures and Data collection.

Aram Mokarizadeh: Immunohistochemical analysis.

Conflicts of interest

None.

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